before taking them to the clean sample preparation facility.

2. Perform sample preparation in a well-equipped clean facility.

Note: The clean area is required to have the following minimum characteristics. The area or hood must be capable of maintaining a positive pressure with make-up air being HEPA filtered. The cumulative analytical blank concentration must average less than 18 s/mm super2 in an area of 0.057 s/mm super2 (nominally 10 200-mesh grid openings) with no more than one single preparation to exceed 53 s/mm super2 for that same area.

3. Preparation areas for air samples must be separated from preparation areas for bulk samples. Personnel must not prepare air samples if they have previously been preparing bulk samples without performing appropriate personal hygiene procedures, i.e., clothing change, showering, etc.

4. Preparation. Direct preparation techniques are required. The objective is to produce an intact carbon film containing the particulates from the filter surface which is sufficiently clear for TEM analysis. Currently recommended direct preparation procedures for polycarbonate (PC) and mixed cellulose ester (MCE) filters are described in Unit III.F.7. and 8. Sample preparation is a subject requiring additional research. Variation on those steps which do not substantively change the procedure, which improve filter clearing or which reduce contamination problems in a laboratory are permitted.

a. Use only TEM grids that have had grid opening areas measured according to directions in Unit III.J.

b. Remove the inlet and outlet plugs prior to opening the cassette to minimize any pressure differential that may be present.

c. Examples of techniques used to prepare polycarbonate filters are described in Unit III.F.7.

d. Examples of techniques used to prepare mixed cellulose ester filters are described in Unit III.F.8.

e. Prepare multiple grids for each sample.

f. Store the three grids to be measured in appropriately labeled grid holders or polyethylene capsules.

5. Equipment.

a. Clean area.

b. Tweezers. Fine-point tweezers for handling of filters and TEM grids.

c. Scalpel Holder and Curved No. 10 Surgical Blades.

d. Microscope slides.

e. Double-coated adhesive tape.

f. Gummed page reinforcements.

g. Micro-pipet with disposal tips 10 to 100 mu L variable volume.

h. Vacuum coating unit with facilities for evaporation of carbon. Use of a liquid nitrogen cold trap above the diffusion pump will minimize the possibility of contamination of the filter surface by oil from the pumping system. The vacuum-coating unit can also be used for deposition of a thin film of gold.

i. Carbon rod electrodes. Spectrochemically pure carbon rods are required for use in the vacuum evaporator for carbon coating of filters.

j. Carbon rod sharpener. This is used to sharpen carbon rods to a neck. The use of necked carbon rods (or equivalent) allows the carbon to be applied to the filters with a minimum of heating.

k. Low-temperature plasma asher. This is used to etch the surface of collapsed mixed cellulose ester (MCE) filters. The asher should be supplied with oxygen, and should be modified as necessary to provide a throttle or bleed valve to control the speed of the vacuum to minimize disturbance of the filter. Some early models of ashers admit air too rapidly, which may disturb particulates on the surface of the filter during the etching step.

l. Glass petri dishes, 10 cm in diameter, 1 cm high. For prevention of excessive evaporation of solvent when these are in use, a good seal must be provided

between the base and the lid. The seal can be improved by grinding the base and lid together with an abrasive grinding material.

m. Stainless steel mesh.

n. Lens tissue.

o. Copper 200-mesh TEM grids, 3 mm in diameter, or equivalent.

p. Gold 200-mesh TEM grids, 3 mm in diameter, or equivalent.

q. Condensation washer.

r. Carbon-coated, 200-mesh TEM grids, or equivalent.

s. Analytical balance, 0.1 mg sensitivity.

t. Filter paper, 9 cm in diameter.

u. Oven or slide warmer. Must be capable of maintaining a temperature of 65- 70 ° C.

v. Polyurethane foam, 6 mm thickness.

w. Gold wire for evaporation.

6. Reagents.

a. General. A supply of ultra-clean, fiber-free water must be available for washing of all components used in the analysis. Water that has been distilled in glass or filtered or deionized water is satisfactory for this purpose. Reagents must be fiber-free.

b. Polycarbonate preparation method—chloroform.

c. Mixed Cellulose Ester (MCE) preparation method—acetone or the Burdette procedure (Ref. 7 of Unit III.L.).

7. TEM specimen preparation from polycarbonate filters.

a. Specimen preparation laboratory. It is most important to ensure that contamination of TEM specimens by extraneous asbestos fibers is minimized during preparation.

b. Cleaning of sample cassettes. Upon receipt at the analytical laboratory and before they are taken into the clean facility or laminar flow hood, the sample cassettes must be cleaned of any contamination adhering to the outside surfaces.

c. Preparation of the carbon evaporator. If the polycarbonate filter has already been carbon-coated prior to receipt, the carbon coating step will be omitted, unless the analyst believes the carbon film is too thin. If there is a need to apply more carbon, the filter will be treated in the same way as an uncoated filter. Carbon coating must be performed with a high-vacuum coating unit. Units that are based on evaporation of carbon filaments in a vacuum generated only by an oil rotary pump have not been evaluated for this application, and must not be used. The carbon rods should be sharpened by a carbon rod sharpener to necks of about 4 mm long and 1 mm in diameter. The rods are installed in the evaporator in such a manner that the points are approximately 10 to 12 cm from the surface of a microscope slide held in the rotating and tilting device.

d. Selection of filter area for carbon coating. Before preparation of the filters, a 75 mm x 50 mm microscope slide is washed and dried. This slide is used to support strips of filter during the carbon evaporation. Two parallel strips of double-sided adhesive tape are applied along the length of the slide. Polycarbonate filters are easily stretched during handling, and cutting of areas for further preparation must be performed with great care. The filter and the MCE backing filter are removed together from the cassette and placed on a cleaned glass microscope slide. The filter can be cut with a curved scalpel blade by rocking the blade from the point placed in contact with the filter. The process can be repeated to cut a strip approximately 3 mm wide across the diameter of the filter. The strip of polycarbonate filter is separated from the corresponding strip of backing filter and carefully placed so that it bridges the gap between the adhesive tape strips on the microscope slide. The filter strip can be held with fine-point tweezers and supported underneath by the scalpel blade during placement on the microscope slide. The analyst can place several such strips on the same microscope slide, taking care to rinse and wet-wipe the scalpel blade and tweezers before handling a new sample. The filter strips should be identified by etching the glass slide or marking the slide using a marker

insoluble in water and solvents. After the filter strip has been cut from each filter, the residual parts of the filter must be returned to the cassette and held in position by reassembly of the cassette. The cassette will then be archived for a period of 30 days or returned to the client upon request.

e. Carbon coating of filter strips. The glass slide holding the filter strips is placed on the rotation-tilting device, and the evaporator chamber is evacuated. The evaporation must be performed in very short bursts, separated by some seconds to allow the electrodes to cool. If evaporation is too rapid, the strips of polycarbonate filter will begin to curl, which will lead to cross-linking of the surface material and make it relatively insoluble in chloroform. An experienced analyst can judge the thickness of carbon film to be applied, and some test should be made first on unused filters. If the film is too thin, large particles will be lost from the TEM specimen, and there will be few complete and undamaged grid openings on the specimen. If the coating is too thick, the filter will tend to curl when exposed to chloroform vapor and the carbon film may not adhere to the support mesh. Too thick a carbon film will also lead to a TEM image that is lacking in contrast, and the ability to obtain ED patterns will be compromised. The carbon film should be as thin as possible and remain intact on most of the grid openings of the TEM specimen intact.

f. Preparation of the Jaffe washer. The precise design of the Jaffe washer is not considered important, so any one of the published designs may be used. A washer consisting of a simple stainless steel bridge is recommended. Several pieces of lens tissue approximately 1.0 cm x 0.5 cm are placed on the stainless steel bridge, and the washer is filled with chloroform to a level where the meniscus contacts the underside of the mesh, which results in saturation of the lens tissue. See References 8 and 10 of Unit III.L.

g. Placing of specimens into the Jaffe washer. The TEM grids are first placed on a piece of lens tissue so that individual grids can be picked up with tweezers. Using a curved scalpel blade, the analyst excises three 3 mm square pieces of the carbon-coated polycarbonate filter from the filter strip. The three squares are selected from the center of the strip and from two points between the outer periphery of the active surface and the center. The piece of filter is placed on a TEM specimen grid with the shiny side of the TEM grid facing upwards, and the whole assembly is placed boldly onto the saturated lens tissue in the Jaffe washer. If carbon-coated grids are used, the filter should be placed carbon-coated side down. The three excised squares of filters are placed on the same piece of lens tissue. Any number of separate pieces of lens tissue may be placed in the same Jaffe washer. The lid is then placed on the Jaffe washer, and the system is allowed to stand for several hours, preferably overnight.

h. Condensation washing. It has been found that many polycarbonate filters will not dissolve completely in the Jaffe washer, even after being exposed to chloroform for as long as 3 days. This problem becomes more serious if the surface of the filter was overheated during the carbon evaporation. The presence of undissolved filter medium on the TEM preparation leads to partial or complete obscuration of areas of the sample, and fibers that may be present in these areas of the specimen will be overlooked; this will lead to a low result. Undissolved filter medium also compromises the ability to obtain ED patterns. Before they are counted, TEM grids must be examined critically to determine whether they are adequately cleared of residual filter medium. It has been found that condensation washing of the grids after the initial Jaffe washer treatment, with chloroform as the solvent, clears all residual filter medium in a period of approximately 1 hour. In practice, the piece of lens tissue supporting the specimen grids is transferred to the cold finger of the condensation washer, and the washer is operated for about 1 hour. If the specimens are cleared satisfactorily by the Jaffe washer alone, the condensation washer step may be unnecessary.

8. TEM specimen preparation from MCE filters.

a. This method of preparing TEM specimens from MCE filters is similar to that specified in NIOSH Method 7402. See References 7, 8, and 9 of Unit III.L.

b. Upon receipt at the analytical laboratory, the sample cassettes must be cleaned of any contamination adhering to the outside surfaces before entering the clean sample preparation area.

c. Remove a section from any quadrant of the

sample and blank filters.

d. Place the section on a clean microscope slide. Affix the filter section to the slide with a gummed paged reinforcement or other suitable means. Label the slide with a water and solvent-proof marking pen.

e. Place the slide in a petri dish which contains several paper filters soaked with 2 to 3 mL acetone. Cover the dish. Wait 2 to 4 minutes for the sample filter to fuse and clear.

f. Plasma etching of the collapsed filter is required.

i. The microscope slide to which the collapsed filter pieces are attached is placed in a plasma asher. Because plasma ashers vary greatly in their performance, both from unit to unit and between different positions in the asher chamber, it is difficult to specify the conditions that should be used. This is one area of the method that requires further evaluation. Insufficient etching will result in a failure to expose embedded filters, and too much etching may result in loss of particulate from the surface. As an interim measure, it is recommended that the time for ashing of a known weight of a collapsed filter be established and that the etching rate be calculated in terms of micrometers per second. The actual etching time used for a particular asher and operating conditions will then be set such that a 1-2 mu m (10 percent) layer of collapsed surface will be removed.

ii. Place the slide containing the collapsed filters into a low-temperature plasma asher, and etch the filter.

g. Transfer the slide to a rotating stage inside the bell jar of a vacuum evaporator. Evaporate a 1 mm x 5 mm section of graphite rod onto the cleared filter. Remove the slide to a clean, dry, covered petri dish.

h. Prepare a second petri dish as a Jaffe washer with the wicking substrate prepared from filter or lens paper placed on top of a 6 mm thick disk of clean spongy polyurethane foam. Cut a V-notch on the edge of the foam and filter paper. Use the V-notch as a reservoir for adding solvent. The wicking substrate should be thin enough to fit into the petri dish without touching the lid.

i. Place carbon-coated TEM grids face up on the filter or lens paper. Label the grids by marking with a pencil on the filter paper or by putting registration marks on the petri dish lid and marking with a waterproof marker on the dish lid. In a fume hood, fill the dish with acetone until the wicking substrate is saturated. The level of acetone should be just high enough to saturate the filter paper without creating puddles.

j. Remove about a quarter section of the carbon-coated filter samples from the glass slides using a surgical knife and tweezers. Carefully place the section of the filter, carbon side down, on the appropriately labeled grid in the acetone-saturated petri dish. When all filter sections have been transferred, slowly add more solvent to the wedge-shaped trough to bring the acetone level up to the highest possible level without disturbing the sample preparations. Cover the petri dish. Elevate one side of the petri dish by placing a slide under it. This allows drops of condensed solvent vapors to form near the edge rather than in the center where they would drip onto the grid preparation.

G. TEM Method

1. Instrumentation.

a. Use an 80-120 kV TEM capable of performing electron diffraction with a fluorescent screen inscribed with calibrated gradations. If the TEM is equipped with EDXA it must either have a STEM attachment or be capable of producing a spot less than 250 nm in diameter at crossover. The microscope shall be calibrated routinely (see Unit III.J.) for magnification and camera constant.

b. While not required on every microscope in the laboratory, the laboratory must have either one microscope equipped with energy dispersive X-ray analysis or access to an equivalent system on a TEM in another laboratory. This must be an Energy Dispersive X-ray Detector mounted on TEM column and associated hardware/software to collect, save, and read out spectral information. Calibration of Multi-Channel Analyzer shall be checked regularly for Al at 1.48 KeV and Cu at 8.04 KeV, as well as the manufacturer's procedures.

i. Standard replica grating may be used to determine magnification (e.g., 2160 lines/mm).

ii. Gold standard may be used to determine camera constant.

c. Use a specimen holder with single tilt and/or double tilt capabilities.

2. Procedure.

a. Start a new Count Sheet for each sample to be analyzed. Record on count sheet: analyst's initials and date; lab sample number; client sample number microscope identification; magnification for analysis; number of predetermined grid openings to be analyzed; and grid identification. See the following Figure 4:

FIGURE 4--COUNT SHEET

Lab Sample No. _____ Filter Type _____ Operator _____
Client Sample No. _____ Filter Area _____ Date _____
Instrument I.D. _____ Grid I.D. _____ Comments _____
Magnification _____ Grid Opening (GO) Area _____
Acc. Voltage _____ No. GO to be Analyzed _____

GO	Structure No.	Structure Type*	Length		ED Observation				EDAX
			< 5µm	≥ 5 µm	Chrys.	Amph.	Nonasb.	Neg. ID	

GO	Structure No.	Structure Type*	Length		ED Observation				EDAX
			< 5µm	≥ 5 µm	Chrys.	Amph.	Nonasb.	Neg. ID	

*B = Bundle
C = Cluster
F = Fiber
M = Matrix

NFD = No fibers detected
N = No diffraction obtained

b. Check that the microscope is properly aligned and calibrated according to the manufacturer's specifications and instructions.

c. Microscope settings: 80-120 kV, grid assessment 250-1000X, then 15,000- 20,000X screen magnification for analysis.

d. Approximately one-half (0.5) of the predetermined sample area to be analyzed shall be performed on one sample grid preparation and the remaining half on a second sample grid preparation.

e. Determine the suitability of the grid.

i. Individual grid openings with greater than 5 percent openings (holes) or covered with greater than 25 percent particulate matter or obviously having nonuniform loading shall not be analyzed.

ii. Examine the grid at low magnification (less than 1000X) to determine its suitability for detailed

study at higher magnifications.

iii. Reject the grid if:

(1) Less than 50 percent of the grid openings covered by the replica are intact.

(2) It is doubled or folded.

(3) It is too dark because of incomplete dissolution of the filter.

iv. If the grid is rejected, load the next sample grid.

v. If the grid is acceptable, continue on to Step 6 if mapping is to be used; otherwise proceed to Step 7.

f. Grid Map (Optional).

i. Set the TEM to the low magnification mode.

ii. Use flat edge or finder grids for mapping.

iii. Index the grid openings (fields) to be counted by marking the acceptable fields for one-half (0.5) of the area needed for analysis on each of the two grids to be analyzed. These may be marked just before examining each grid opening (field), if desired.

iv. Draw in any details which will allow the grid to be properly oriented if it is reloaded into the microscope and a particular field is to be reliably identified.

g. Scan the grid.

i. Select a field to start the examination.

ii. Choose the appropriate magnification (15,000 to 20,000X screen magnification).

iii. Scan the grid as follows.

(1) At the selected magnification, make a series of parallel traverses across the field. On reaching the end of one traverse, move the image one window and reverse the traverse.

Note: A slight overlap should be used so as not to miss any part of the grid opening (field).

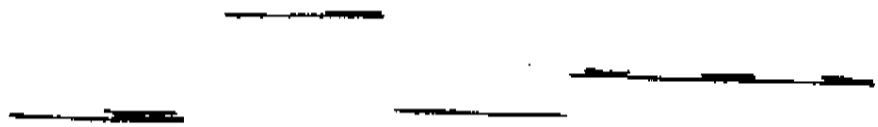
(2) Make parallel traverses until the entire grid opening (field) has been scanned.

h. Identify each structure for appearance and size.

i. Appearance and size: Any continuous grouping of particles in which an asbestos fiber within aspect ratio greater than or equal to 5:1 and a length greater than or equal to 0.5 mu m is detected shall be recorded on the **count** sheet. These will be designated asbestos structures and will be classified as fibers, bundles, clusters, or matrices. Record as individual fibers any contiguous grouping having 0, 1, or 2 definable intersections. Groupings having more than 2 intersections are to be described as cluster or matrix. See the following Figure 5:

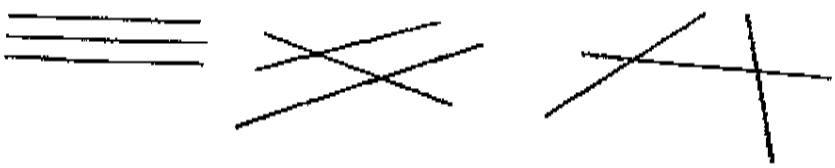
FIGURE 5--COUNTING GUIDELINES USED IN DETERMINING ASBESTOS STRUCTURES

Count as 1 fiber; 1 structure; no intersections.

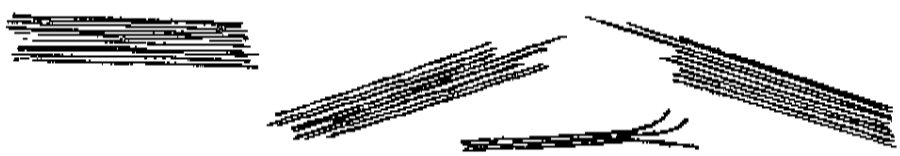


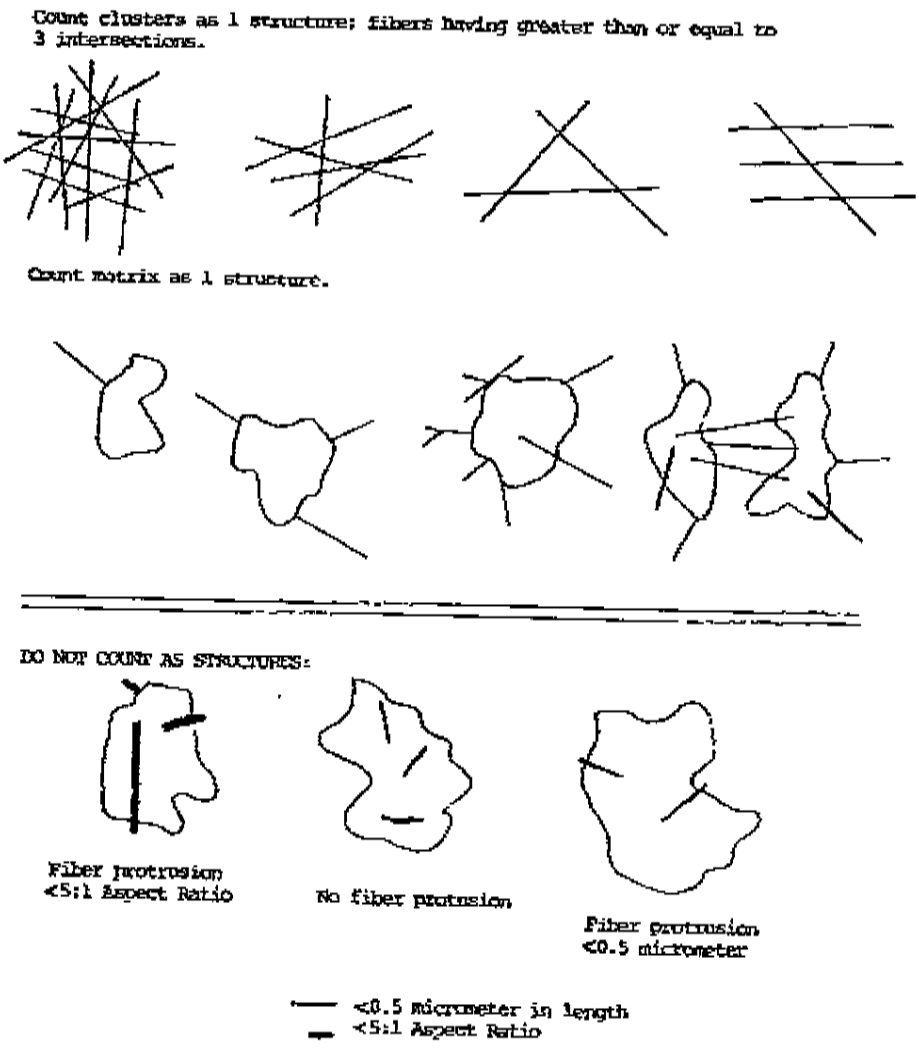
Count as 2 fibers if space between fibers is greater than width of 1 fiber diameter or number of intersections is equal to or less than 1.

Count as 3 structures if space between fibers is greater than width of 1 fiber diameter or if the number of intersections is equal to or less than 2.



Count bundles as 1 structure; 3 or more parallel fibrils less than 1 fiber diameter separation.





An intersection is a non-parallel touching or crossing of fibers, with the projection having an aspect ratio of 5:1 or greater. Combinations such as a matrix and cluster, matrix and bundle, or bundle and cluster are categorized by the dominant fiber quality--cluster, bundle, and matrix, respectively. Separate categories will be maintained for fibers less than 5 mu m and for fibers greater than or equal to 5 mu m in length. Not required, but useful, may be to record the fiber length in 1 mu m intervals. (Identify each structure morphologically and analyze it as it enters the "window".)

(1) Fiber. A structure having a minimum length greater than 0.5 mu m and an aspect ratio (length to width) of 5:1 or greater and substantially parallel sides. Note the appearance of the end of the fiber, i.e., whether it is flat, rounded or dovetailed, no intersections.

(2) Bundle. A structure composed of 3 or more fibers in a parallel arrangement with each fiber closer than one fiber diameter.

(3) Cluster. A structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group; groupings

must have more than 2 intersections.

(4) Matrix. Fiber or fibers with one end free and the other end embedded in or hidden by a particulate. The exposed fiber must meet the fiber definition.

(5) NSD. Record NSD when no structures are detected in the field.

(6) Intersection. Non-parallel touching or crossing of fibers, with the projection having an aspect ratio 5:1 or greater.

ii. Structure Measurement.

(1) Recognize the structure that is to be sized.

(2) Memorize its location in the "window" relative to the sides, inscribed square and to other particulates in the field so this exact location can be found again when scanning is resumed.

(3) Measure the structure using the scale on the screen.

(4) Record the length category and structure type classification on the **count** sheet after the field number and fiber number.

(5) Return the fiber to its original location in the window and scan the rest of the field for other fibers; if the direction of travel is not remembered, return to the right side of the field and begin the traverse again.

i. Visual identification of Electron Diffraction (ED) patterns is required for each asbestos structure counted which would cause the analysis to exceed the 70 s/mm super2 concentration. (Generally this means the first four fibers identified as asbestos must exhibit an identifiable diffraction pattern for chrysotile or amphibole.)

i. Center the structure, focus, and obtain an ED pattern. (See Microscope Instruction Manual for more detailed instructions.)

ii. From a visual examination of the ED pattern, obtained with a short camera length, classify the observed structure as belonging to one of the following classifications: chrysotile, amphibole, or nonasbestos.

(1) Chrysotile: The chrysotile asbestos pattern has characteristic streaks on the layer lines other than the central line and some streaking also on the central line. There will be spots of normal sharpness on the central layer line and on alternate lines (2nd, 4th, etc.). The repeat distance between layer lines is 0.53 nm and the center doublet is at 0.73 nm. The pattern should display (002), (110), (130) diffraction maxima; distances and geometry should match a chrysotile pattern and be measured semiquantitatively.

(2) Amphibole Group [includes grunerite (amosite), crocidolite, anthophyllite, tremolite, and actinolite]: Amphibole asbestos fiber patterns show layer lines formed by very closely spaced dots, and the repeat distance between layer lines is also about 0.53 nm. Streaking in layer lines is occasionally present due to crystal structure defects.

(3) Nonasbestos: Incomplete or unobtainable ED patterns, a nonasbestos EDXA, or a nonasbestos morphology.

iii. The micrograph number of the recorded diffraction patterns must be reported to the client and maintained in the laboratory's quality assurance records. The records must also demonstrate that the identification of the pattern has been verified by a qualified individual and that the operator who made the identification is maintaining at least an 80 percent correct visual identification based on his measured patterns. In the event that examination of the pattern by the qualified individual indicates that the pattern had been misidentified visually, the client shall be contacted. If the pattern is a suspected chrysotile, take a photograph of the diffraction pattern at 0 degrees tilt. If the structure is suspected to be amphibole, the sample may have to be tilted to obtain a simple geometric array of spots.

j. Energy Dispersive X-Ray Analysis (EDXA).

i. Required of all amphiboles which would cause the analysis results to exceed the 70 s/mm super2 concentration. (Generally speaking, the first 4 amphiboles would require EDXA.)

ii. Can be used alone to confirm chrysotile after the 70 s/mm super2 concentration has been exceeded.

iii. Can be used alone to confirm all nonasbestos.

iv. Compare spectrum profiles with profiles obtained from asbestos standards. The closest match identifies and categorizes the structure.

v. If the EDXA is used for confirmation, record the properly labeled spectrum on a computer disk, or if a hard copy, file with analysis data.

vi. If the number of fibers in the nonasbestos class would cause the analysis to exceed the 70 s/mm super2 concentration, their identities must be confirmed by EDXA or measurement of a zone axis diffraction pattern to establish that the particles are nonasbestos.

k. Stopping Rules.

i. If more than 50 asbestiform structures are counted in a particular grid opening, the analysis may be terminated.

ii. After having counted 50 asbestiform structures in a minimum of 4 grid openings, the analysis may be terminated. The grid opening in which the 50th fiber was counted must be completed.

iii. For blank samples, the analysis is always continued until 10 grid openings have been analyzed.

iv. In all other samples the analysis shall be continued until an analytical sensitivity of 0.005 s/cm super3 is reached.

l. Recording Rules. The **count** sheet should contain the following information:

i. Field (grid opening): List field number.

ii. Record "NSD" if no structures are detected.

iii. Structure information.

(1) If fibers, bundles, clusters, and/or matrices are found, list them in consecutive numerical order, starting over with each field.

(2) Length. Record length category of asbestos fibers examined. Indicate if less than 5 mu m or greater than or equal to 5 mu m.

(3) Structure Type. Positive identification of asbestos fibers is required by the method. At least one diffraction pattern of each fiber type from every five samples must be recorded and compared with a standard diffraction pattern. For each asbestos fiber reported, both a morphological descriptor and an identification descriptor shall be specified on the **count** sheet.

(4) Fibers classified as chrysotile must be identified by diffraction and/or X-ray analysis and recorded on the **count** sheet. X-ray analysis alone can be used as sole identification only after 70s/mm super2 have been exceeded for a particular sample.

(5) Fibers classified as amphiboles must be identified by X-ray analysis and electron diffraction and recorded on the **count** sheet. (X-ray analysis alone can be used as sole identification only after 70s/mm super2 have been exceeded for a particular sample.)

(6) If a diffraction pattern was recorded on film, the micrograph number must be indicated on the **count** sheet.

(7) If an electron diffraction was attempted and an appropriate spectra is not observed, N should be recorded on the **count** sheet.

(8) If an X-ray analysis is attempted but not observed, N should be recorded on the **count** sheet.

(9) If an X-ray analysis spectrum is stored, the file and disk number must be recorded on the **count** sheet.

m. Classification Rules.

i. Fiber. A structure having a minimum length greater than or equal to 0.5 mu m and an aspect ratio (length to width) of 5:1 or greater and substantially parallel sides. Note the appearance of the end of the fiber, i.e., whether it is flat, rounded or dovetailed.

ii. Bundle. A structure composed of three or more fibers in a parallel arrangement with each fiber closer than one fiber diameter.

iii. Cluster. A structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group. Groupings must have more than two intersections.

iv. Matrix. Fiber or fibers with one end free and the other end embedded in or hidden by a particulate. The exposed fiber must meet the fiber definition.

v. NSD. Record NSD when no structures are detected in the field.

n. After all necessary analyses of a particle structure have been completed, return the goniometer stage to 0 degrees, and return the structure to its original location by recall of the original location.

o. Continue scanning until all the structures are identified, classified and sized in the field.

p. Select additional fields (grid openings) at low magnification; scan at a chosen magnification (15,000 to 20,000X screen magnification); and analyze until the stopping rule becomes applicable.

q. Carefully record all data as they are being collected, and check for accuracy.

r. After finishing with a grid, remove it from the microscope, and replace it in the appropriate grid hold. Sample grids must be stored for a minimum of 1 year from the date of the analysis; the sample cassette must be retained for a minimum of 30 days by the laboratory or returned at the client's request.

H. Sample Analytical Sequence

1. Carry out visual inspection of work site prior to air monitoring.

2. Collect a minimum of five air samples inside the work site and five samples outside the work site. The indoor and outdoor samples shall be taken during the same time period.

3. Analyze the abatement area samples according to this protocol. The analysis must meet the 0.005 s/cm super3 analytical sensitivity.

4. Remaining steps in the analytical sequence are contained in Unit IV. of this Appendix.

I. Reporting

The following information must be reported to the client. See the following Table II:

TABLE II—EXAMPLE LABORATORY LETTERHEAD

Laboratory I.D.	Client I.D.	FILTER MEDIA DATA				Analyzed Area, mm^2	Sample Volume, cc
		Type	Diameter, mm	Effective Area, mm^2	Pore Size, µm		

INDIVIDUAL ANALYTICAL RESULTS

Laboratory I.D.	Client I.D.	# Asbestos Structures	Analytical Sensitivity, str/cc	CONCENTRATION	
				Structures/mm^2	Structures/cc

The analysis was carried out to the approved TEM method. This laboratory is in compliance with the quality specified by the method.

Authorized Signature

1. Concentration in structures per square millimeter and structures per cubic centimeter.

2. Analytical sensitivity used for the analysis.

3. Number of asbestos structures.

4. Area analyzed.

5. Volume of air samples (which was initially provided by client).

6. Average grid size opening.

7. Number of grids analyzed.

8. Copy of the **count** sheet must be included with the report.

9. Signature of laboratory official to indicate that the laboratory met specifications of the AHERA method.

10. Report form must contain official laboratory identification (e.g., letterhead).

11. Type of asbestos.

J. Calibration Methodology

Note: Appropriate implementation of the method requires a person knowledgeable in electron diffraction and mineral identification by ED and EDXA. Those inexperienced laboratories wishing to develop capabilities may acquire necessary knowledge through analysis of appropriate standards and by following detailed methods as described in References 8 and 10 of Unit III.L.

1. Equipment Calibration. In this method, calibration is required for the air-sampling equipment and the transmission electron microscope (TEM).

a. TEM Magnification. The magnification at the fluorescent screen of the TEM must be calibrated at the grid opening magnification (if used) and also at the magnification used for fiber counting. This is performed with a cross grating replica. A logbook must be maintained, and the dates of calibration depend on the past history of the particular microscope; no frequency is specified. After any maintenance of the microscope that involved adjustment of the power supplied to the lenses or the high-voltage system or the mechanical disassembly of the electron optical column apart from filament exchange, the magnification must be recalibrated. Before the TEM calibration is performed, the analyst must ensure that the cross grating replica is placed at the same distance from the objective lens as the specimens are. For instruments that incorporate an eucentric tilting specimen stage, all specimens and the cross grating replica must be placed at the eucentric position.

b. Determination of the TEM magnification on the fluorescent screen.

i. Define a field of view on the fluorescent screen either by markings or physical boundaries. The field of view must be measurable or previously inscribed with a scale or concentric circles (all scales should be metric).

ii. Insert a diffraction grating replica (for example a grating containing 2,160 lines/mm) into the specimen holder and place into the microscope. Orient the replica so that the grating lines fall perpendicular to the scale on the TEM fluorescent screen. Ensure that the goniometer stage tilt is 0 degrees.

iii. Adjust microscope magnification to 10,000X or 20,000X. Measure the distance (mm) between two widely separated lines on the grating replica. Note the number of spaces between the lines. Take care to measure between the same relative positions on the lines (e.g., between left edges of lines).

Note: The more spaces included in the measurement, the more accurate the final calculation. On most microscopes, however, the magnification is substantially constant only within the central 8-10 cm diameter region of the fluorescent screen.

iv. Calculate the true magnification (M) on the fluorescent screen:

M=XG/Y

where:

X=total distance (mm) between the designated grating lines;

G=calibration constant of the grating replica (lines/mm):

Y=number of grating replica spaces counted along X.

c. Calibration of the EDXA System. Initially, the EDXA system must be calibrated by using two reference elements to calibrate the energy scale of the instrument. When this has been completed in accordance with the manufacturer's instructions, calibration in terms of the different types of asbestos can proceed. The EDXA detectors vary in both solid angle of detection and in window thickness. Therefore, at a particular accelerating voltage in use on the TEM, the count rate obtained from specific dimensions of fiber will vary both in absolute X-ray count rate and in the relative X-ray peak heights for different elements. Only a few minerals are relevant for asbestos abatement work,

and in this procedure the calibration is specified in terms of a "fingerprint" technique. The EDXA spectra must be recorded from individual fibers of the relevant minerals, and identifications are made on the basis of semiquantitative comparisons with these reference spectra.

d. Calibration of Grid Openings.

i. Measure 20 grid openings on each of 20 random 200-mesh copper grids by placing a grid on a glass slide and examining it under the PCM. Use a calibrated graticule to measure the average field diameter and use this number to calculate the field area for an average grid opening. Grids are to be randomly selected from batches up to 1,000.

Note: A grid opening is considered as one field.

ii. The mean grid opening area must be measured for the type of specimen grids in use. This can be accomplished on the TEM at a properly calibrated low magnification or on an optical microscope at a magnification of approximately 400X by using an eyepiece fitted with a scale that has been calibrated against a stage micrometer. Optical microscopy utilizing manual or automated procedures may be used providing instrument calibration can be verified.

c. Determination of Camera Constant and ED Pattern Analysis.

i. The camera length of the TEM in ED operating mode must be calibrated before ED patterns on unknown samples are observed. This can be achieved by using a carbon-coated grid on which a thin film of gold has been sputtered or evaporated. A thin film of gold is evaporated on the specimen TEM grid to obtain zone-axis ED patterns superimposed with a ring pattern from the polycrystalline gold film.

ii. In practice, it is desirable to optimize the thickness of the gold film so that only one or two sharp rings are obtained on the superimposed ED pattern. Thicker gold film would normally give multiple gold rings, but it will tend to mask weaker diffraction spots from the unknown fibrous particulates. Since the unknown d-spacings of most interest in asbestos analysis are those which lie closest to the transmitted beam, multiple gold rings are unnecessary on zone- axis ED patterns. An average camera constant using multiple gold rings can be determined. The camera constant is one-half the diameter, D, of the rings times the interplanar spacing, d, of the ring being measured.

K. Quality Control/Quality Assurance Procedures (Data Quality Indicators)

Monitoring the environment for airborne asbestos requires the use of sensitive sampling and analysis procedures. Because the test is sensitive, it may be influenced by a variety of factors. These include the supplies used in the sampling operation, the performance of the sampling, the preparation of the grid from the filter and the actual examination of this grid in the microscope. Each of these unit operations must produce a product of defined quality if the analytical result is to be a reliable and meaningful test result. Accordingly, a series of control checks and reference standards is performed along with the sample analysis as indicators that the materials used are adequate and the operations are within acceptable limits. In this way, the quality of the data is defined and the results are of known value. These checks and tests also provide timely and specific warning of any problems which might develop within the sampling and analysis operations. A description of these quality control/quality assurance procedures is summarized in the following Table III:

TABLE III--SUMMARY OF LABORATORY DATA QUALITY OBJECTIVES

Unit Operation	QC Check	Frequency	Conformance Expectation
Sample receiving	Review of receiving report	Each sample	95% complete
Sample custody	Review of chain-of-custody record	Each sample	95% complete
Sample preparation	Supplies and reagents	On receipt	Meet specs. or reject
	Grid opening size	20 openings/20 grids/lot of 1000 or 1 opening/sample	100%
	Special clean area monitoring	After cleaning or service	Meet specs. or reclean
	Laboratory blank	1 per prep series or 10%	Meet specs. or reanalyze series
	Plasma etch blank	1 per 20 samples	75%
	Multiple preps (3 per sample)	Each sample	One with cover of 15 complete grid sqs.
Sample analysis	System check	Each day	Each day
	Alignment check	Each day	Each day
	Magnification calibration with low and high standards	Each month or after service	95%
	ED calibration by gold standard	Weekly	95%
	EDS calibration by copper line	Daily	95%
Performance check	Laboratory blank (measure of cleanliness)	Prep 1 per series or 10% read 1 per 25 samples	Meet specs. or reanalyze series
	Replicate counting (measure of precision)	1 per 100 samples	1.5 x Poisson Std. Dev.
	Duplicate analysis (measure of reproducibility)	1 per 100 samples	2 x Poisson Std. Dev.
	Known samples of typical materials (working standards)	Training and for comparison with unknowns	100%
	Analysis of NBS SRM 1876 and/or RM 8410 (measure of accuracy and comparability)	1 per analyst per year	1.5 x Poisson Std. Dev.
	Data entry review (data validation and measure of completeness)	Each sample	95%

	Record and verify ID electron diffraction pattern of structure	1 per 5 samples	80% accuracy
Calculations and data reduction	Hand calculation of automated data reduction procedure or independent recalculation of hand-calculated data	1 per 100 samples	85%

1. When the samples arrive at the laboratory, check the samples and documentation for completeness and requirements before initiating the analysis.

2. Check all laboratory reagents and supplies for acceptable asbestos background levels.

3. Conduct all sample preparation in a clean room environment monitored by laboratory blanks and special testing after cleaning or servicing the room.

4. Prepare multiple grids of each sample.

5. Provide laboratory blanks with each sample batch. Maintain a cumulative average of these results. If this average is greater than 53 f/mm super2 per 10 200-mesh grid openings, check the system for possible sources of contamination.

6. Check for recovery of asbestos from cellulose ester filters submitted to plasma asher.

7. Check for asbestos carryover in the plasma asher by including a blank alongside the positive control sample.

8. Perform a systems check on the transmission electron microscope daily.

9. Make periodic performance checks of magnification, electron diffraction and energy dispersive X-ray systems as set forth in Table III of Unit III.K.

10. Ensure qualified operator performance by evaluation of replicate counting, duplicate analysis, and standard sample comparisons as set forth in Table III of Unit III.K.

11. Validate all data entries.

12. Recalculate a percentage of all computations and automatic data reduction steps as specified in Table III.

13. Record an electron diffraction pattern of one asbestos structure from every five samples that contain asbestos. Verify the identification of the pattern by measurement or comparison of the pattern with patterns collected from standards under the same conditions.

The outline of quality control procedures presented above is viewed as the minimum required to assure that quality data is produced for clearance testing of an asbestos abated area. Additional information may be gained by other control tests. Specifics on those control procedures and options available for environmental testing can be obtained by consulting References 6, 7, and 11 of Unit III.L.

L. References

For additional background information on this method the following references should be consulted.

1. "Guidelines for Controlling Asbestos-Containing Materials in Buildings," EPA 56 0/5-85-024, June 1985.

2. "Measuring Airborne Asbestos Following an Abatement Action," USEPA/ Office of Pollution Prevention and Toxics, EPA 600/4-85-049, 1985.

3. Small, John and E. Steel. Asbestos Standards: Materials and Analytical Methods. N.B.S. Special Publication 619, 1982.

4. Campbell, W.J., R.L. Blake, L.L. Brown, E.E. Cather, and J.J. Sjoberg. Selected Silicate Minerals and Their Asbestiform Varieties. Information Circular 8751, U.S. Bureau of Mines, 1977.

5. Quality Assurance Handbook for Air Pollution Measurement System. Ambient Air Methods, EPA 600/4-77-027a, USEPA, Office of Research and

Development, 1977.

6. Method 2A: Direct Measurement of Gas Volume Through Pipes and Small Ducts. 40 CFR Part 60 Appendix A.

7. Burdette, G.J. Health & Safety Exec., Research & Lab. Services Div. , London, "Proposed Analytical Method for Determination of Asbestos in Air."

8. Chatfield, E.J., Chatfield Tech. Cons., Ltd., Clark, T., PEI Assoc. "Standard Operating Procedure for Determination of Airborne Asbestos Fibers by Transmission Electron Microscopy Using Polycarbonate Membrane Filters." WERL SOP 87-1, March 5, 1987.

9. NIOSH. Method 7402 for Asbestos Fibers, December 11, 1986 Draft.

10. Yamate, G., S.C. Agarwall, R.D. Gibbons, IIT Research Institute, "Methodology for the Measurement of Airborne Asbestos by Electron Microscopy." Draft report, USEPA Contract 68-02-3266, July 1984.

11. Guidance to the Preparation of Quality Assurance Project Plans. USEPA, Office of Pollution Prevention and Toxics, 1984.

IV. Mandatory Interpretation of Transmission Electron Microscopy Results to Determine Completion of Response Actions

A. Introduction

A response action is determined to be completed by TEM when the abatement area has been cleaned and the airborne asbestos concentration inside the abatement area is no higher than concentrations at locations outside the abatement area. "Outside" means outside the abatement area, but not necessarily outside the building. EPA reasons that an asbestos removal contractor cannot be expected to clean an abatement area to an airborne asbestos concentration that is lower than the concentration of air entering the abatement area from outdoors or from other parts of the building. After the abatement area has passed a thorough visual inspection, and before the outer containment barrier is removed, a minimum of five air samples inside the abatement area and a minimum of five air samples outside the abatement area must be collected. Hence, the response action is determined to be completed when the average airborne asbestos concentration measured inside the abatement area is not statistically different from the average airborne asbestos concentration measured outside the abatement area.

The inside and outside concentrations are compared by the Z-test, a statistical test that takes into account the variability in the measurement process. A minimum of five samples inside the abatement area and five samples outside the abatement area are required to control the false negative error rate, i.e., the probability of declaring the removal complete when, in fact, the air concentration inside the abatement area is significantly higher than outside the abatement area. Additional quality control is provided by requiring three blanks (filters through which no air has been drawn) to be analyzed to check for unusually high filter contamination that would distort the test results.

When volumes greater than or equal to 1,199 L for a 25 mm filter and 2,799 L for a 37 mm filter have been collected and the average number of asbestos structures on samples inside the abatement area is no greater than 70 s/mm super2 of filter, the response action may be considered complete without comparing the inside samples to the outside samples. EPA is permitting this initial screening test to save analysis costs in situations where the airborne asbestos concentration is sufficiently low so that it cannot be distinguished from the filter contamination/background level (fibers deposited on the filter that are unrelated to the air being sampled). The screening test cannot be used when volumes of less than 1,199 L for 25 mm filter or 2,799 L for a 37 mm filter are collected because the ability to distinguish levels significantly different from filter background is reduced at low volumes.

The initial screening test is expressed in structures per square millimeter of filter because filter background levels come from sources other than the air being sampled and cannot be meaningfully expressed as a concentration per cubic centimeter of air. The value of 70 s/mm super2 is based on the experience of the panel of microscopists who consider one structure in 10 grid openings (each

grid opening with an area of 0.0057 mm super2) to be comparable with contamination/background levels of blank filters. The decision is based, in part, on Poisson statistics which indicate that four structures must be counted on a filter before the fiber **count** is statistically distinguishable from the **count** for one structure. As more information on the performance of the method is collected, this criterion may be modified. Since different combinations of the number and size of grid openings are permitted under the TEM protocol, the criterion is expressed in structures per square millimeter of filter to be consistent across all combinations. Four structures per 10 grid openings corresponds to approximately 70 s/mm super2 .

B. Sample Collection and Analysis

1. A minimum of 13 samples is required: five samples collected inside the abatement area, five samples collected outside the abatement area, two field blanks, and one sealed blank.

2. Sampling and TEM analysis must be done according to either the mandatory or nonmandatory protocols in Appendix A. At least 0.057 mm super2 of filter must be examined on blank filters.

C. Interpretation of Results

1. The response action shall be considered complete if either:

a. Each sample collected inside the abatement area consists of at least 1,199 L of air for a 25 mm filter, or 2,799 L of air for a 37 mm filter, and the arithmetic mean of their asbestos structure concentrations per square millimeter of filter is less than or equal to 70 s/mm (superscript 2); or

b. The three blank samples have an arithmetic mean of the asbestos structure concentration on the blank filters that is less than or equal to 70 s/mm super2 and the average airborne asbestos concentration measured inside the abatement area is not statistically higher than the average airborne asbestos concentration measured outside the abatement area as determined by the Z-test. The Z-test is carried out by calculating

$$Z = \frac{\bar{Y}_1 - \bar{Y}_0}{0.8(1/n_1 + 1/n_0)^{1/2}}$$

where Y subI is the average of the natural logarithms of the inside samples and Y subO) is the average of the natural logarithms of the outside samples, n subI is the number of inside samples and n subO is the number of outside samples. The response action is considered complete if Z is less than or equal to 1.65.

Note: When no fibers are counted, the calculated detection limit for that analysis is inserted for the concentration.

2. If the abatement site does not satisfy either (1) or (2) above, the site must be recleaned and a new set of samples collected.

D. Sequence for Analyzing Samples

It is possible to determine completion of the response action without analyzing all samples. Also, at any point in the process, a decision may be made to terminate the analysis of existing samples, reclean the abatement site, and collect a new set of samples. The following sequence is outlined to minimize the number of analyses needed to reach a decision.

1. Analyze the inside samples.

2. If at least 1,199 L of air for a 25 mm filter or 2,799 L of air for a 37 mm filter is collected for each inside sample and the arithmetic mean concentration of structures per square millimeter of filter is less than or equal to 70 s/mm super2 , the response action is complete and no further analysis is needed.

3. If less than 1,199 L of air for a 25 mm filter or 2,799 L of air for a 37 mm filter is collected for any of the inside samples, or the arithmetic mean concentration of structures per square millimeter of filter is greater than 70 s/mm super2 , analyze the three blanks.

4. If the arithmetic mean concentration of structures per square millimeter on the blank filters is greater than 70 s/mm super2 , terminate the analysis, identify and correct the source of blank contamination, and collect a new set of samples.

5. If the arithmetic mean concentration of structures per square millimeter on the blank filters is less than or equal to 70 s/mm super2 , analyze the outside samples and perform the Z-test.

6. If the Z-statistic is less than or equal to 1.65, the response action is complete. If the Z-statistic is greater than 1.65, reclean the abatement site and collect a new set of samples.

[52 FR 41857, Oct. 30, 1987]

<General Materials (GM) - References, Annotations, or Tables>

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